Mentype® **AMLplex**QS
Manual

Novel detection of chromosomal aberrations in acute myeloid leukemia

In-Vitro-Diagnostics

CE IVD

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25
100
400

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Made in Germany

Bio type® Diagnostic GmbH

Biotype Diagnostic GmbH develops, produces and markets their PCR-based rapid Mentype® Detection Kits. Our products provide customers with fast and reliable testing methods for professional medical diagnostics.

Our Mentype® Test Kits guarantee highest quality standards for clinical research and diagnostics.

For information and enquiries about the Mentype® **AMLplex**^{QS} PCR Amplification Kit, please do not hesitate to get in touch or visit www.biotype.de/en/home.html.

Product description

The verification of specific chromosomal aberrations has high prognostic value in nearly all types of acute leukemia. Molecular biological evidence of chromosomal aberrations (translocations) represents an important diagnostic completion. Detecting specific translocations enables subtype-classification of leukemic diseases and provides essential information for the risk-directed therapy of patients.

Mentype® **AMLplex**QS facilitates detection of the most common chromosomal aberrations yet observed in acute myeloid leukemia (AML) and represents a simple-to-use, routine-fit and reliable screening tool.

Mentype® **AMLplex**QS contains optimised reagents for high resolution detection of 11 fusion gene transcripts (AML1-ETO, BCR-ABL, CALM-AF10, CBFB-MYH11, DEK-CAN, MLL-AF6, MLL-AF9, MLL-ELL, MLL-PTD, NPM1-MLF1 and PML-RARA) with 34 transcript variants in total (Table1).
The test is performed by fragment analysis using capillary gel electrophoresis. One primer for each transcript is fluorescence-labelled with 6-FAM, BTG, BTY.

The test kit includes an internal PCR-Control (Quality Sensor “QS-Control”) and a “cDNA Control” (ABL-Control) providing helpful information about PCR efficiency, quality of applied cDNA templates, and presence of PCR-inhibitors.

Mentype® **AMLplex**QS was validated and evaluated for GeneAmp® 9700 Silver Thermocycler, Eppendorf Mastercycler ep-S, Biometra T1, ABI PRISM® 310 Genetic Analyzer running POP4® and ABI PRISM® 3130 Genetic Analyzer running POP4®.
The development, manufacture and distribution of Biotype® products are certified according to DIN EN ISO 9001:2008.

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1. Description of the Mentype® AMLplex^{QS}

Table 1. Detected Chromosomal Aberrations and Variants

Gene-Fusions	Chromosomal Aberration	Variants
AML1-ETO	t(8;21) (q22;q22)	-
BCR-ABL	t(9;22) (q34;q11)	e1a3
		e1a2
		b3a2
		b3a3
		b2a2
		b2a3
CALM-AF10	t(10;11) (p13;q14)	AF10_240-CALM_1987
		AF10_240-CALM_2092
CBFB-MYH11	inv(16) (p13;q22)	Type A
		Type B
		Type C
		Type D
		Type E
		Type F
		Type G
		Type H
		Type I
		Type J
DEK-CAN	t(6;9) (p23;q34)	-
MLL-AF6	t(6;11) (q27;q23)	-
MLL-AF9	t(9;11) (p22;q23)	6A_(THP-1)
		7A_(10A)
		8A_(MM6)
		6B_(9B)
MLL-ELL	t(11;19) (q23;p13.1)	e10e2
		e10e3
MLL-PTD	Partial Tandem Duplication	e9e3
		e10e3
		e11e3
NPM1-MLF1	t(3;5) (q25.1;q34)	-
PML-RARA	t(15;17) (q22;q21)	bcr1 (PR-L)
		bcr2 (PR-V)
		bcr3 (PR-S)

Table 2. Quality Sensors of the Mentype® **AMLplex**^{QS}

Quality Sensors	Meaning
Internal PCR-Control (QS-Control)	Reflects quality of PCR performance
cDNA-Control (ABL-Control)	Reflects quality of cDNA templates

Kit content

Mentype® **AMLplex**^{QS} PCR Amplification Kit (100 Reactions)

Nuclease-free water	3.0 ml
Reaction mix **A**	500 µl
Primer mix	250 µl
Multi Taq2 DNA Polymerase	40 µl
Control cDNA KASUMI-1 (500 ng/µl)	10 µl
DNA Size Standard 550 (BTO)	50 µl
Allelic ladder	25 µl

Ordering information

Mentype® **AMLplex**^{QS}	25	reactions	Cat.No.	45-31220-0025
Mentype® **AMLplex**^{QS}	100	reactions	Cat.No.	45-31220-0100
Mentype® **AMLplex**^{QS}	400	reactions	Cat.No.	45-31220-0400

Storage

Store all components at -20 °C and avoid repeated thawing and freezing. Primer mix and allelic ladder must be stored protected from light. The DNA samples and post-PCR reagents (allelic ladder and DNA size standard) should be stored separately from PCR reagents. The expiry date is indicated on the kit cover.

Additionally required reagents

Additional reagents are needed in order to use the Biotype® PCR Amplification Kit:

Reagent	**Supplier**	**Order number**
Hi-Di™ Formamide, 25 ml	Life Technologies Corporation	4311320
Matrix Standards BT5 single-capillary instruments (5x25 µl)	Biotype Diagnostic GmbH	00-10411-0025
Matrix Standards BT5 multi-capillary instruments (25 µl)	Biotype Diagnostic GmbH	00-10421-0025
Matrix Standards BT5 multi-capillary instruments (50 µl)	Biotype Diagnostic GmbH	00-10421-0050

Warnings and safety instructions

The PCR Amplification Kit contains the following potentially hazardous chemicals:

Kit component	Chemical	Hazards
Reaction mix	Sodium azide NaN_3	toxic if swallowed, develops toxic gases when it gets in contact with acids

Observe the Material Safety Data Sheets (MSDS) for all Biotype® products, which are available on request. Please contact the respective manufacturers for copies of the MSDS for any additionally needed reagents.

Quality assurance

All kit components undergo an intensive quality assurance process at Biotype Diagnostic GmbH. The quality of the test kits is permanently monitored in order to ensure unrestricted usability. Please contact us if you have any questions regarding quality assurance.

Trademarks and Patents

Mentype® is a registered trademark of Biotype Diagnostic GmbH.
ABI PRISM®, GeneMapper®, GeneAmp® and Applied Biosystems® are registered trademarks of Applied Biosystems LLC.
Under the law of Europe POP-4® is a registered trademark of Applied Biosystems LLC.
POP-4® is registered as trademark of Life Technologies Corporation in the US.
The PCR is covered by patents. Patentees are Hoffmann-La Roche Inc. and F. Hoffmann-La Roche (Roche).

2. Outline of required working stages

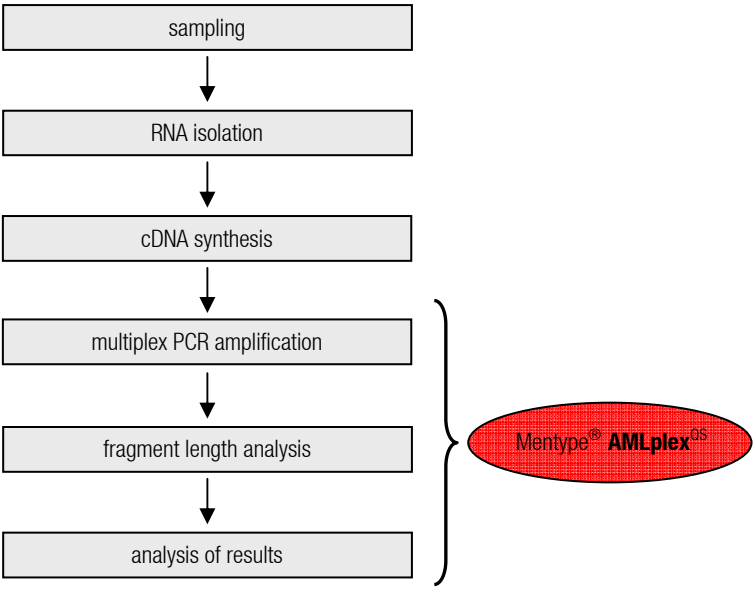


Fig. 1 From sample to analysis – detection of fusion gene transcripts performed with the Mentype® **AMLplex**QS PCR Amplification Kit

Protocols for amplification, electrophoresis and analysis

3. PCR amplification

3.1 Master Mix preparation

The table below shows volumes of applied reagents per 1.0 µl sample volume (template-cDNA) in a total reaction volume of 25 µl. The number of reactions to be set up shall be determined taking into account positive and negative control reactions. Add one or two reactions to this number to compensate pipetting errors.

Component	Volume
Nuclease-free water	16.1 µl
Reaction mix **A***	5.0 µl
Primer mix	2.5 µl
Multi Taq2 DNA Polymerase (hot start, 2.5 U/µl)	0.4 µl
Volume of master mix	24.0 µl

* contains Mg^{2+}, dNTPs, BSA

All components should be mixed (vortex) and centrifuged for about 10 s before preparing the master mix.

Since performance of the Mentype® **AMLplex**^{QS} analysis is mostly depending on quality and quantity of applied cDNA, we recommend standardised and already validated methods for sampling, RNA isolation and RNA to cDNA transcription e.g. of the Europe Against Cancer Program (EAC, see references p.33).

The amount of cDNA used for the assay depends on the concentration and quality of prior isolated and applied RNA. For reference samples generated from cell culture the use of 1 µl cDNA will be sufficient if 1 µg of respective RNA was initially transcribed in a RT-PCR reaction volume of 20 µl. The amount of applied template can be extended in case of critical clinical samples. Adjust the final reaction volume to 25 µl with nuclease-free water.

The primer mix is optimised to result in sufficient peak-heights if **25 PCR cycles** in 25µl reaction volume are performed. The ABL-Control should not exceed the specified measuring range of the used instrument herein.

Positive control

Dilute Control cDNA KASUMI-1 to 250 ng/µl in appropriate volume. Instead of template cDNA pipette diluted Control cDNA into reaction-tubes containing the PCR master mix.

Negative control

Nuclease-free water serves as negative control. Pipette respective volume instead of the cDNA template into reaction tubes containing the PCR master mix.

Template cDNA

Sometimes, measured value of the cDNA concentration varies depending on the quantification method used. In this instant it may be necessary to adjust the optimal cDNA amount.

3.2 PCR amplification parameter

Perform a “hot start” PCR in order to activate the Multi Taq2 DNA Polymerase and to prevent the formation of non-specific amplification products.

The number of PCR cycles depends on the amount of applied cDNA. 25 PCR cycles are recommended for all samples. For highly concentrated reference samples from cell cultures a reduction to 22 PCR cycles is recommended. In case of critical samples, we suggest to increase the number of PCR cycles to a maximum of 28 cycles.
The **internal ABL-Control** may serve as point of reference to evaluate the optimal number of required PCR cycles. The optimal range of the internal ABL-Control should not exceed the specified measuring range of the used instrument herein (e.g. 500 to 5000 RFU on ABI3130).

Very small amounts of cDNA may result in statistical dropouts and imbalances of the peaks. Increasing numbers of PCR cycles raise the risk of cross contamination caused by minimal amounts of impurities. Furthermore, unspecific amplification products could appear.

Standard method

Recommended for all cDNA samples

Temperature	Time	
96 °C	4 min (hot start to activate Multi Taq2 DNA Polymerase)	
96 °C	30 s	
61 °C	**120 s**	**25 cycles**
72 °C	75 s	
68 °C	10 min*	
10 °C	∞	hold

Optional

Recommended for cDNA positive controls from cell culture

Temperature	Time	
96 °C	4 min (hot start to activate Multi Taq2 DNA Polymerase)	
96 °C	30 s	
61 °C	**120 s**	**22 cycles**
72 °C	75 s	
68 °C	10 min*	
10 °C	∞	hold

Optional

Recommended for critical cDNA samples

Temperature	Time	
96 °C	4 min (hot start to activate Multi Taq2 DNA Polymerase)	
96 °C	30 s	
61 °C	**120 s**	**max. 28 cycles**
72 °C	75 s	
68 °C	10 min*	
10 °C	∞	hold

* If a higher amount of minus-Adenine peaks is observed, extension up to 60 min is possible.

4. Electrophoresis using the ABI PRISM® 310 Genetic Analyzer

For general instructions on instrument setup, matrix generation and application of the GeneScan or GeneMapper® ID software, please refer to the *ABI PRISM® 310 Genetic Analyzer User's Manual*. Electrophoresis using the GeneScan software is described below.

The virtual **filter set G5** shall be used for combined application of the five fluorescent labels **6-FAM**, **BTG**, **BTY**, **BTR**, **and BTO** (the matrix standard will be called **BT5** hereinafter).

Material	
Capillary	47 cm / 50 µm (green)
Polymer	POP-4® for 310 Genetic Analyzer
Buffer	10x Genetic Analyzer Buffer with EDTA

4.1 Matrix generation

Prior to conducting DNA fragment size analysis with the filter set G5, a matrix with the five fluorescent labels 6-FAM, BTG, BTY, BTR, and BTO must be generated.

Color	**Matrix standard**
Blue (B)	6-FAM
Green (G)	BTG
Yellow (Y)	BTY
Red (R)	BTR
Orange (O)	BTO

Five electrophoresis runs shall be conducted, one for each fluorescent label, 6-FAM, BTG, BTY, BTR, and BTO; use same conditions as applied to generate suitable matrix files for samples and allelic ladders.

Matrix sample	**Component**	**Volume**
Matrix sample 1	Hi-Di™ Formamide	12.0 µl
	Matrix standard **6-FAM**	1.0 µl
Matrix sample 2	Hi-Di™ Formamide	12.0 µl
	Matrix standard **BTG**	1.0 µl
Matrix sample 3	Hi-Di™ Formamide	12.0 µl
	Matrix standard **BTY**	1.0 µl
Matrix sample 4	Hi-Di™ Formamide	12.0 µl
	Matrix standard **BTR**	1.0 µl
Matrix sample 5	Hi-Di™ Formamide	12.0 µl
	Matrix standard **BTO**	1.0 µl

- Denaturation for 3 min at 95 °C
- Cool down to 4 °C and place samples on the autosampler tray

- Create a **Sample Sheet**, choose **5 Dyes** and enter a sample designation

Injection list for matrix generation

Parameter	**Set up**
Module File	GS STR POP-4 (1 ml) **G5**
Matrix File	**NONE**
Size Standard*	**NONE**
Injection [s]	5
Injection [kV]	15.0
Run [kV]	15.0
Run [°C]	60
Run Time [min]	24

* Prepare matrix standards always **without DNA Size Standard (BTO)**

Analysis of the matrix samples

- Run the GeneScan software
- **File** → **New** → **Project** (open folder of current run) → **Add Sample Files**
- Select a matrix sample in the **Sample File** column
- **Sample** → **Raw Data**
- Check the matrix samples for a flat baseline. As shown in the figure below there should be at least five peaks with peak heights about 1000-4000 RFU (Y-axis) for each matrix sample (optimal range: 2000-4000 RFU)

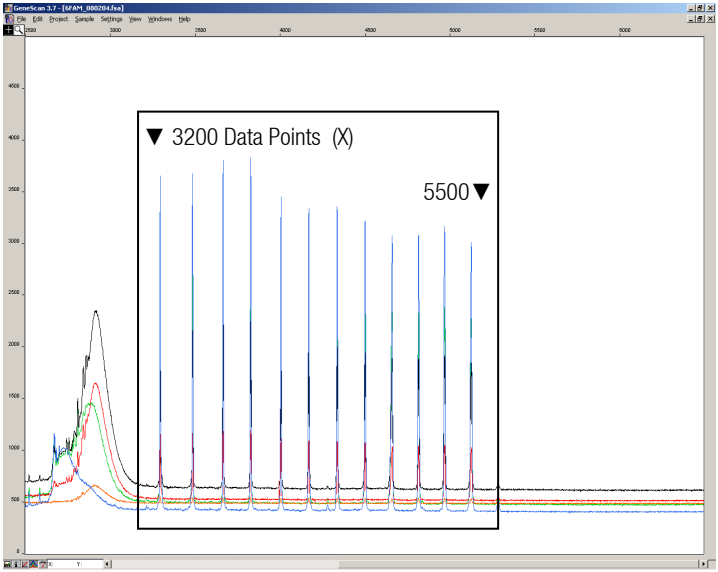


Fig. 2 Electropherogram with raw data of the matrix standard 6-FAM

- Select an analysis range with flat baseline and re-inject the matrix sample if necessary
- Note down start and end value (data points) of the analysis range, e.g. start value 3200, end value 5500
- Calculate the difference, e.g. 5500-3200 = 2300 data points

Generation of a new matrix

- **File** → **New** → **Matrix**

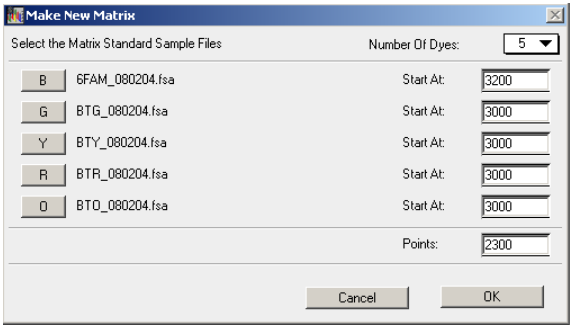


Fig. 3 Matrix sample selection

- Import matrix samples for all dyes (B, G, Y, R, O)
- Enter a **Start At** value, e.g. 3200
- Enter the calculated difference under **Points**, e.g. 2300
- Click on **OK** to calculate the new matrix

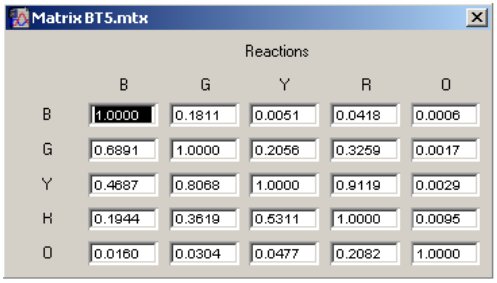


Fig. 4 New matrix BT5

- Save the matrix in the matrix folder: **File** → **Save as**, e.g. Matrix BT5

Matrix check

Check the new matrix with current samples.
- **File** → **New** → **Project** (open folder of the respective run) → **Add Sample Files**
- Select sample(s) in the **Sample File** column
- **Sample** → **Install New Matrix** (open matrix folder and select new matrix)
- Re-analyse your samples

There should be **no** pull-up peaks between the dye panels (B, G, Y, R, O) with the new matrix.

4.2 Sample preparation

Component	Volume
Hi-Di™ Formamide	12.0 µl
DNA Size Standard 550 (BTO)	0.5 µl
prepare 12 µl of the mix (formamide + DNA size standard) for all samples	
add 1 µl PCR product (dilute if necessary) or allelic ladder	

- Denaturation for 3 min at 95 °C
- Cool down to 4 °C and place samples on the autosampler tray

Signal intensities

Options to increase the signal intensity:

- Reduce the volume of the DNA Size Standard 550 (BTO) to peak heights of about 500 relative fluorescent units (RFU)
- Purify the PCR products before starting the analysis

4.3 Setting up the Data Collection Software

- Create a **Sample Sheet** and enter sample designation

Injection list

Parameter	Set up
Module File	GS STR POP-4 (1 ml) **G5**
Matrix File	e.g. Matrix BT5
Size Standard	e.g. SST-BTO_60-550bp
Injection [s]*	**5**
Injection [kV]	15.0
Run [kV]	15.0
Run [°C]	60
Run Time [min]**	**28**

* Deviating from the standard settings, the injection time may range between 1 and 20 s depending on the type of sample. If reference samples with very high signal intensities are recorded, a shorter injection time may be selected in order to avoid pull-up peaks. For samples with low cDNA content or critical patient samples an injection time of up to 20 s may be necessary.

** Depending on the analysis conditions, the run time for Mentype® **AMLplex^{QS}** should be modified in order to analyse fragments with lengths of up to **550 bp**.

4.4 Analysis parameters / analysis method

The recommended analysis parameters are:

Analysis Range	Full range
Data Processing	Baseline: Checked Multicomponent: Checked Smooth Options: Light
Peak Detection	Peak Amplitude Thresholds B:* Y:* G:* R:* O:* Min. Peak Half Width: 2 pts Polynominal Degree: 3 Peak Window Size: 15 pts**
Size Call Range	Min: 60 Max: 550
Size Calling Method	Local Southern Method
Split Peak Correction	None

* The peak amplitude threshold (cut-off value) corresponds to the minimum peak height that will be detected by the GeneScan or GeneMapper® ID software. For the Mentype® **AMLplex^{QS}** 200 RFU is recommended and should be determined individually by the laboratory. Recommendation: The minimal peak height should be three times as high as the background noise of the baseline.

** If necessary, Peak Window Size can be minimised to 11 pts to improve peak detection.

5. Electrophoresis using the ABI PRISM® 3100-Avant/3100 Genetic Analyzer

For detailed instructions on instrument setup, spectral calibration, application of the Data Collection software and the GeneScan software, please refer to the *ABI PRISM® 3100-Avant/3100 Genetic Analyzer User's Manual*. This chapter describes the use of ABI PRISM® 3100-Avant/3100 Genetic Analyzer in combination with Data Collection software version 1.0.1 and 1.1. For systems with Data Collection software 2.0 or 3.0 refer to chapter 6.
The system with 4 capillaries is named ABI 3100-Avant, and the system with 16 capillaries is named ABI 3100.

The virtual **filter set G5** shall be used for combined application of the five fluorescent labels **6-FAM**, **BTG**, **BTY**, **BTR and BT0** (the matrix standard will be called **BT5** hereinafter).

Material

Capillary*	36 cm Capillary Array for 3100-Avant/3100
Polymer*	POP-4® Polymer for 3100
Buffer	10x Genetic Analyzer Buffer with EDTA

*other instrument settings possible

5.1 Spectral calibration / matrix generation

Proper spectral calibration is critical to evaluate multicolor systems with the ABI PRISM® 3100-Avant/3100 Genetic Analyzer and shall be done prior to conducting fragment length analysis. The calibration procedure creates a matrix that is used to correct the overlap of fluorescence emission spectra of the dyes.

Spectral calibration comprises the following steps:
- Preparation of spectral calibration standards
- Loading standards to the 96-well reaction plate (one sample per capillary)
- Entering the plate composition
- Performing a spectral calibration run and checking the matrix

Setting up the spectral calibration standard

Example for 4 capillaries / ABI 3100-Avant

Component	**Volume**
Hi-Di™ Formamide	60.0 µl
Matrix standard **BT5**	5.0 µl

- Load 12 µl of the mix to a 96-well reaction plate, e.g. position **A1-D1**
- Denaturation for 3 min at 95 °C
- Cool down to 4 °C and place samples on the autosampler tray

Example for 16 capillaries / ABI 3100

Component	**Volume**
Hi-Di™ Formamide	204.0 µl
Matrix standard **BT5**	17.0 µl

- Load 12 µl of the mix to a 96-well reaction plate, e.g. position **A1-H1** and **A2-H2**
- Denaturation for 3 min at 95 °C
- Cool down to 4 °C and place samples on the autosampler tray

Performing a spectral calibration run

First of all, the parameter file for **DyeSetG5** must be modified once to achieve successful calibration with the Data Collection software version 1.0.1 or 1.1.

Spectral parameter

To change settings in the parameter file go to the following path:
D:\AppliedBio\Support Files\Data Collection Support Files\CalibrationData\Spectral Calibration\ParamFiles

- Select **MtxStd{Genescan_SetG5}** to open the PAR-file
- Change **Condition Bounds Range** to [1.0; 20.0]
- Select **File** → **Save As** to save the parameter file under a new name, e.g. MtxStd{Genescan_SetG5_BT5}.par

Always use this parameter file for spectral calibration runs using Biotype® matrix standard **BT5**.

Plate Editor for spectral calibration (I)

- Place the 96-well plate on the autosampler tray
- Run the ABI PRISM® 3100 Data Collection software
- In **Plate View** click **New** to open the **Plate Editor** dialog box
- Enter a name of the plate
- Select **Spectral Calibration**
- Select **96-Well** as plate type and click on **Finish**

Plate editor for spectral calibration (II)

Parameter	**Set up**
Sample Name	Enter name for the matrix samples
Dye Set	G5
Spectral Run Module	*Default* (e.g. Spect36_POP4)
Spectral Parameters	MtxStd{GeneScan_SetG5_BT5}.par (parameters created before)

- Click into the column header to select the entire column, select **Edit** → **Fill Down** to apply the information of the selected samples and confirm with **OK**
- Link your reaction plate on the autosampler tray with the created plate ID and start the run
- On completion of the run check in the **Spectral Calibration Result** dialog box if all capillaries have successfully passed calibration (label **A**). If individual capillaries are labelled **X**, refer to *ABI PRISM® Genetic Analyzer User's Manual*
- Click on **OK** to confirm completion of the run

Matrix check

- Select **Tools → Display Spectral Calibration → Dye Set → G5** to review the spectral calibration profile for each capillary
- The quality value (**Q value**) must be greater than 0.95 and the condition number (**C value**) must be between 1 and 20. Both values must be within the previously determined range
- Check the matrix samples for a flat baseline. There should be five peaks with peak heights of about 1000-5000 RFU (Y-axis) in each matrix sample (optimal range: 2000-4000 RFU)
- Check the new matrix with your current samples. There should be **no** pull-up peaks between the dye panels (B, G, Y, R, O) with the new matrix
- If calibration was not successful, try to re-inject the samples with higher injection voltage or injection time. The editing of the Spectral Run Module will be necessary. You can re-inject the same samples up to three times. Otherwise use more matrix standard for spectral calibration
- If all capillaries have passed the calibration, the last calibration file for **Dye Set G5** must be activated manually under **Tools → Set Active Spectral Calibration**. Rename the calibration file under **Set Matrix Name** (e.g. BT5_Date of calibration)

5.2 Sample preparation

Component	Volume
Hi-Di™ Formamide	12.0 µl
DNA Size Standard 550 (BTO)	0.5 µl
Prepare 12 µl of the mix (formamide + DNA size standard) for all samples	
Add 1 µl PCR product (dilute if necessary) or allelic ladder	

- Denaturation for 3 min at 95 °C
- Cool down to 4 °C and place samples on the autosampler tray

Since injections take place simultaneously on all capillaries, 4 or 16 samples must be pipetted on the plate of multi-capillary analyzers. If fewer samples are analysed, the empty positions must be filled with 12 µl Hi-Di™ Formamide.

Run several ladders to ensure a reliable allelic assignment on multi-capillary analyzers.

Room temperature may influence the performance of PCR products on multi-capillary instruments, so that shoulder peaks or split peaks occur especially at low temperatures. Pay attention to keep ambient conditions as recommended by the instrument manufacturer. Optimal settings were reported >22 °C room temperature.

Signal intensities

Options to increase the signal intensity:

- Reduce the volume of the DNA Size Standard 550 (BTO) to peak heights of about 500 relative fluorescent units (RFU)
- Purify the PCR products before starting the analysis

5.3 Setting up the Data Collection Software

Edit the default run module in **Dye Set G5** once for the first run.
- Select **Module Editor** to open the dialog box
- Select the appropriate **Run Module** as template from the **GeneScan** table
- Modify the **Injection Voltage** to 3 kV and the **Injection Time** to 10 s

Run Module 3kV_10s_550bp

Parameter	**Set up**
Run Temperature [°C]	*Default*
Cap Fill Volume	*Default*
Maximum Current [A]	*Default*
Current Tolerance [A]	*Default*
Run Current [A]	*Default*
Voltage Tolerance [kV]	*Default*
Pre Run Voltage [kV]	*Default*
Pre Run Time [s]	*Default*
Injection Voltage [kV]	**3.0**
Injection Time [s]*	**10**
Run Voltage [kV]	*Default*
Number of Steps	*Default*
Voltage Step Interval	*Default*
Data Delay Time [s]	*Default*
Run Time [min]**	**26**

* Deviating from the standard settings, the injection time may range between 1 and 20 s depending on the type of sample. If reference samples with very high signal intensities are recorded, a shorter injection time may be selected in order to avoid pull-up peaks. For samples with low DNA content or critical patient samples an injection time of up to 20 s may be necessary.

** Depending on the analysis conditions, the run time for Mentype® **AMLplex^{QS}** should be modified in order to analyse fragments with lengths of up to **550 bp**.

- Click on **Save As**, enter the name of the new module (e.g. 3kV_10s_550bp) and confirm with **OK**
- Click on **Close** to exit the **Run Module Editor**

Starting the run

- Place the prepared 96-well plate on the autosampler tray
- Run the ABI PRISM® 3100 Data Collection software
- In **Plate View** click on **New** to open the Plate Editor dialog box
- Enter a name of the plate
- Select **GeneScan**
- Select **96-Well** as plate type and click on **Finish**

Plate Editor

Parameter	**Set up**
Sample Name	Enter name for the samples
Dyes	0
Color Info	Ladder or sample
Project Name	e.g. 3100_Project1
Dye Set	G5
Run Module*	3kV_10s_550bp
Analysis Module 1	DefaultAnalysis.gsp

* parameter see above

- Complete the table in the **Plate Editor** and click on **OK**
- Click into the column header to select the entire column and select **Edit → Fill Down** to apply the information of the selected samples
- Link your reaction plate on the autosampler tray with the created plate ID and start the run
- On completion of the run, view data as **Color Data** in **Array View** of the 3100 Data Collection software or as **Analyzed Sample Files** under D:/AppliedBio/3100/DataExtractor/ExtractRuns

5.4 Analysis parameters / analysis method

The recommended analysis parameters are:

Analysis Range	Full range
Data Processing	Baseline: Checked Multicomponent: Checked Smooth Options: Light
Peak Detection	Peak Amplitude Thresholds B:* Y:* G:* R:* O:* Min. Peak Half Width: 2 pts Polynominal Degree: 3 Peak Window Size: 15 pts**
Size Call Range	Min: 60 Max: 550
Size Calling Method	Local Southern Method
Split Peak Correction	None

* The peak amplitude threshold (cut-off value) corresponds to the minimum peak height that will be detected by the GeneScan or GeneMapper® ID software. For the Mentype® **AMLplex**^{QS} 200 RFU is recommended and should be determined individually by the laboratory. Recommendation: The minimal peak height should be three times as high as the background noise of the baseline

** If necessary, Peak Window Size can be minimised to 11 pts to improve peak detection.

6. Electrophoresis using the ABI PRISM® 3130/3130xl Genetic Analyzer

For detailed instructions on instrument setup, spectral calibration, or application of the ABI PRISM® Data Collection software version 3.0 and the GeneMapper® ID/ID-X software, refer to the *ABI PRISM® 3130/3130xl Genetic Analyzers Getting Started Guide*.

The system with 4 capillaries is named ABI 3130 and the system with 16 capillaries is named ABI 3130xl.

The virtual **filter set Any5Dye** shall be used for the combined application of the five fluorescent labels **6-FAM**, **BTG**, **BTY**, **BTR**, **and BTO** (the matrix standard will be called **BT5** hereinafter).

Material

Capillary*	36 cm Capillary Array for 3130/3130xl
Polymer*	POP-4® Polymer for 3130
Buffer	10x Genetic Analyzer Buffer with EDTA

* other instrument settings possible

6.1 Spectral calibration / matrix generation

Prior to conducting DNA fragment size analysis, it is necessary to perform a spectral calibration with the four fluorescent labels 6-FAM, BTG, BTY, BTR, and BTO for each analyzer. The calibration procedure creates a matrix which is used to correct the overlap of fluorescence emission spectra of the dyes.

Spectral calibration comprises the following steps:
- Preparation of spectral calibration standards
- Loading the standards to the 96-well reaction plate (one sample per capillary)
- Creating the instrument protocol for spectral calibration (Protocol Manager)
- Defining the plate composition in the plate editor (Plate Manager)
- Performing a spectral calibration run and checking the matrix

Setting up the spectral calibration standards

Example for 4 capillaries / ABI 3130

Component	Volume
Hi-Di™ Formamide	60.0 µl
Matrix standard **BT5**	5.0 µl

- Load 12 µl of the mix to a 96-well reaction plate, e.g. position **A1-D1**
- Denaturation for 3 min at 95 °C
- Cool down to 4 °C and place samples on the autosampler tray

Example for16 capillaries / ABI 3130xl

Component	Volume
Hi-Di™ Formamide	204.0 µl
Matrix standard **BT5**	17.0 µl

- Load 12 µl of the mix to a 96-well reaction plate, e.g. position **A1-H1** and **A2-H2**
- Denaturation for 3 min at 95 °C
- Cool down to 4 °C and place samples on the autosampler tray

Performing a spectral calibration run

- Place the 96-well plate on the autosampler tray
- In the **Protocol Manager** of the Data Collection software click on **New** in **Instrument Protocol** to open the **Protocol Editor** dialog box

Instrument Protocol for spectral calibration

Protocol Editor	Set up
Name	*User* (e.g. Spectral36_POP4_BT5)
Type	SPECTRAL
Dye Set	**Any5Dye**
Polymer*	*User* (e.g. POP4)
Array Length*	*User* (e.g. 36cm)
Chemistry	Matrix Standard
Run Module*	*Default* (e.g. Spect36_POP4_1)

* Depends on the type of polymer and length of capillary used

- Click on **OK** to leave the **Protocol Editor** dialog box
- In the **Plate Manager** of the Data Collection software, click on **New** to open the **New Plate Dialog** box

Plate Editor for spectral calibration (I)

New Plate Dialog	Set up
Name	e.g. Spectral_BT5_date
Application	Spectral Calibration
Plate Type	96-Well
Owner Name / Operator Name	…

- Click on **OK**. A new table in the **Plate Editor** will open automatically

Plate Editor for spectral calibration (II)

Parameter	**Set up**
Sample Name	Enter name for the matrix samples
Priority	e.g. 100
Instrument Protocol 1	Spectral36_POP4_BT5 (setting described before)

- Click into the column header to select the entire column, select **Edit → Fill Down** to apply the information to all selected samples, and click on **OK**
- In the **Run Scheduler** click on **Find All**, select **Link** to link the reaction plate on the autosampler to the newly created plate record (position A or B), and, start the run.

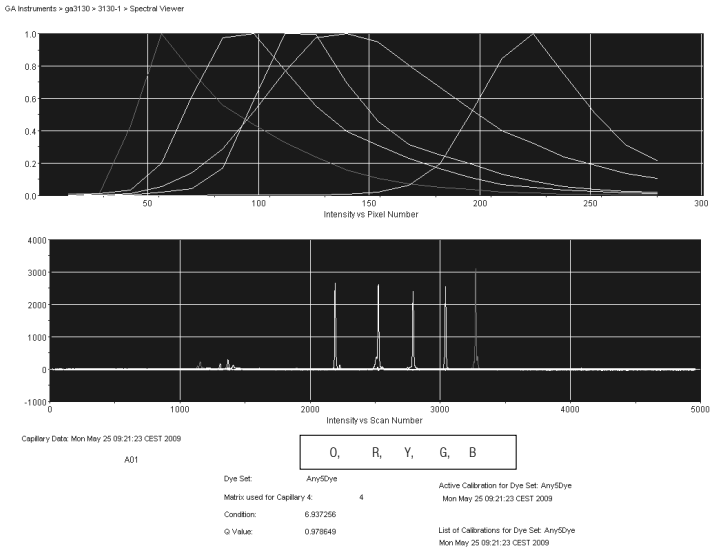

GA Instruments > ga3130 > 3130-1 > Spectral Viewer
Intensity vs Pixel Number
Intensity vs Scan Number
Capillary Data: Mon May 25 09:21:23 CEST 2009
A01
O, R, Y, G, B
Dye Set: Any5Dye
Matrix used for Capillary 4: 4
Condition: 6.937256
Q Value: 0.978649
Active Calibration for Dye Set: Any5Dye
Mon May 25 09:21:23 CEST 2009
List of Calibrations for Dye Set: Any5Dye
Mon May 25 09:21:23 CEST 2009

Fig. 5 Electropherogram of spectral calibration with matrix standard BT5 on an ABI 3130

Matrix check

- The quality value (**Q value**) of each capillary must be greater than 0.95 and the condition number range (**C value**) must be between 1 and 20
- Check the matrix samples for a flat baseline. As shown in the figure above, there should be five peaks with peak heights of about 1000-5000 RFU (Y-axis) in each matrix sample (optimal range: 2000-4000 RFU)
- Check the new matrix with your current samples. There should be **no** pull-up peaks between the dye panels (B, G, Y, R, O) with the new matrix
- If all capillaries have passed the test, the last calibration file for the Dye Set **Any5Dye** is activated automatically in the **Spectral Viewer**. **Rename** the calibration file (e.g. BT5_Date of calibration) using the respective button
- If calibration was not successful, try to re-inject the samples with higher injection voltage or injection time. Editing of the Spectral Run Module will be necessary. You can re-inject the same samples up to three times. Otherwise use more matrix standard for spectral calibration

6.2 Sample preparation

Components	Volume
Hi-Di™ Formamide	12.0 µl
DNA Size Standard 550 (BTO)	0.5 µl
prepare 12 µl of the mix (formamide + DNA size standard) for all samples	
add 1 µl PCR product (diluted if necessary) or allelic ladder	

- Denaturation for 3 min at 95 °C
- Cool down to 4 °C and place samples on the autosampler tray

Since injections take place simultaneously on all capillaries, 4 or 16 samples must be pipetted on the plate of multi-capillary analyzers. If fewer samples are analysed, the empty positions must be filled with 12 µl Hi-Di™ Formamide.

Run several ladders to ensure a reliable allelic assignment on multi-capillary analyzers.

Room temperature may influence the performance of PCR products on multi-capillary instruments. Shoulder peaks or split peaks could occur especially at low temperatures. Pay attention to keep ambient conditions as recommended by the instrument manufacturer. Optimal settings were reported >22 °C room temperature.

Signal intensities

Options to increase the signal intensity:

- Reduce the volume of the DNA Size Standard 550 (BTO) to peak heights of about 500 relative fluorescent units (RFU)
- Purify the PCR products before starting the analysis

6.3 Setting up the Data Collection Software

Edit the Run Module as follows for the first run:

- In the **Module Manager** of the Data Collection software click on **New** to open the **Run Module Editor** dialog box

Run Module 3kV_10s_550bp

Parameter	**Set up**
Oven Temperature [°C]	*Default*
Poly Fill Volume	*Default*
Current Stability [µA]	*Default*
PreRun Voltage [kV]	*Default*
PreRun Time [s]	*Default*
Injection Voltage [kV]	**3.0**
Injection Time [s]*	**10**
Voltage Number of Steps	*Default*
Voltage Step Interval	*Default*
Data Delay Time [s]	*Default*
Run Voltage [kV]	*Default*
Run Time [s]**	**1560**

* Deviating from the standard settings, the injection time may range between 1 and 20 s depending on the type of sample. If reference samples with very high signal intensities are recorded, a shorter injection time may be selected in order to avoid pull-up peaks. For samples with low DNA content or critical patient samples an injection time of up to 20 s may be necessary.

** Depending on the analysis conditions, the run time for Mentype® **AMLplex^{QS}** should be modified in order to analyse fragments with lengths of up to **550 bp**.

- Click on **Save As**, enter the name of the new module (e.g. 3kV_10s_550bp) and confirm with **OK**
- Click on **Close** to exit the **Run Module Editor**

Starting the run

- Place the prepared 96-well plate on the autosampler tray
- In the **Protocol Manager** of the Data Collection software, click on **New** in the **Instrument Protocol** window to open the **Protocol Editor** dialog box

Instrument Protocol

Protocol Editor	**Set up**
Name	e.g. Run36_POP4_BT5_26min
Type	REGULAR
Run Module*	3kV_10s_550bp
Dye Set	**Any5Dye**

* parameter see above

- Click on **OK** to exit the **Protocol Editor**

Prior to each run, it is necessary to create a plate definition as follows:

- In the **Plate Manager** of the Data Collection software click on **New** to open the **New Plate Dialog** box

Plate Editor (I)

New Plate Dialog	**Set up**
Name	e.g. Plate_BT5_Date
Application	Select GeneMapper Application
Plate Type	96-Well
Owner Name / Operator Name	…

- Click on **OK**. A new table in the **Plate Editor** will open automatically

Plate Editor (II)

Parameter	**Set up**
Sample Name	Enter name for the samples
Priority	e.g. 100 (Default)
Sample Type	Sample or allelic ladder
Size Standard	e.g. SST-BTO_60-550bp
Panel	e.g. AMLplex_Panels_v1
Analysis Method	e.g. AMLplex_HID_3130_200rfu
Snp Set	-
User-defined 1-3	-
Results Group 1	(select results group)
Instrument Protocol 1	Run36_POP4_BT5_26min (setting described before)

- Click into the column header to select the entire column, select **Edit → Fill Down** to apply the information to all selected samples and click on **OK**
- In the **Run Scheduler,** click on **Find All**, select **Link** to link the reaction plate on the autosampler to the newly created plate record (position A or B) and start the run
- During the run, view **Error Status** in the **Event Log** or examine the quality of the raw data for each capillary in the **Capillaries Viewer** or the **Cap/Array Viewer**
- View data as overview in **Run History** or **Cap/Array Viewer** of the Data Collection software. Run data are saved in the **Run Folder** of the previously chosen **Result Group**

6.4 Analysis parameters / analysis method

The recommended analysis parameters are:

Peak Detection Algorithm	Advanced
Ranges	Analysis: Full Range Sizing: All Sizes
Smoothing and Baselining	Smoothing: Light Baseline Window: 51 pts
Size Calling Method	Local Southern Method
Peak Detection	Peak Amplitude Thresholds B:* Y:* G:* R:* O:* Min. Peak Half Width: 2 pts Polynominal Degree: 3 Peak Window Size: 15 pts** Slope Thresholds: 0.0

* The peak amplitude threshold (cut-off value) corresponds to the minimum peak height that will be detected by the GeneMapper® ID software. For the Mentype® **AMLplex**^{QS} 200 RFU is recommended and should be determined individually by the laboratory. Recommendation: The minimal peak height should be three times as high as the background noise of the baseline.

** If necessary, Peak Window Size can be minimised to 11 pts to improve peak detection.

7. Electrophoresis using the ABI PRISM® 3500/3500xL Genetic Analyzer

For detailed instructions on instrument setup, spectral calibration, or application of the Applied Biosystems 3500 Series Data Collection Software version 1.0 and the GeneMapper® ID-X software version 1.2, refer to the *Applied Biosystems 3500/3500xL Genetic Analyzers* User Guide.

The system with 8 capillaries is named AB 3500 and the system with 24 capillaries is named AB 3500xL.

The virtual **filter set Any5Dye** shall be used for the combined application of five fluorescent labels **6-FAM**, **BTG**, **BTY**, **BTR**, **and BTO** (the matrix standard will be called **BT5** hereinafter).

Material

Capillary*	36 cm Capillary Array for 3500/3500xL
Polymer	POP-4® Polymer for 3500/3500xL
Buffer	10x Genetic Analyzer Buffer with EDTA for 3500/3500xL

* other instrument settings possible

7.1 Spectral calibration / matrix generation

Prior to conducting DNA fragment size analysis, it is necessary to perform a spectral calibration with the fluorescent labels 6-FAM, BTG, BTY, BTR, and BTO for each analyzer. The calibration procedure creates a matrix that is used to correct the overlap of fluorescence emission spectra of the dyes.

Spectral calibration comprises the following steps:
- Preparation of spectral calibration standards
- Loading the standards to the multi-well reaction plate (one sample per capillary)
- Preparation of instrument and creating a Dye Set BT5
- Performing a spectral calibration run and checking the matrix

Setting up the spectral calibration standards

Example for 8 capillaries / ABI 3500

Component	Volume
Hi-Di™ Formamide	108.0 µl
Matrix standard **BT5**	9.0 µl

- Load 12 µl of the mix to a 96-well reaction plate, e.g. position **A1-H1**
- Denaturation for 3 min at 95°C
- Cool down to 4°C and place samples on the autosampler tray

Example for 24 capillaries / ABI 3500xL

Component	Volume
Hi-Di™ Formamide	300.0 µl
Matrix standard **BT5**	25.0 µl

- Load 12 µl of the mix to a 96-well reaction plate, e.g. position **A1-H1**, **A2-H2 and A3-H3***
- Denaturation for 3 min at 95°C
- Cool down to 4°C and place samples on the autosampler tray

* When using a 384-well plate, load 10 µl of the mixtures to columns 1, 3, and 5 in rows A, C, E, G, I, K, M, and O.

Performing a spectral calibration run

- Place the multi-well plate on the autosampler tray
- Now prepare the instrument and specific spectral calibration run settings

Preparation of the instrument

Before starting the spectral calibration process ensure that the spatial calibration has been performed. This process is necessary if a new capillary array was installed before and is described in detail in the *Applied Biosystems 3500/3500xL Genetic Analyzers User Guide*.

Preparation of dye set BT5

Prior to the spectral calibration, a dye set for the matrix standard BT5 needs to be setup.

1. To create a new dye set, go to **Library** and select **Analyze**, followed by **Dye Sets** and click **Create**.
2. Enter a **Dye Set Name**, e.g. BT5.
3. Select **Matrix Standard** as a chemistry and **AnyDye Template** as a dye set template.
4. Disable **Purple** in the field **Arrange Dyes.** Ensure that all other colors are enabled.
5. Under **Calibration Peak Order** the colors need to be arranged as follows: **5 – blue, 4 – green, 3 – yellow, 2 – red, and 1 – orange**.
6. Do not alter the **Parameter** settings.
7. Click **Save** to confirm the changes.

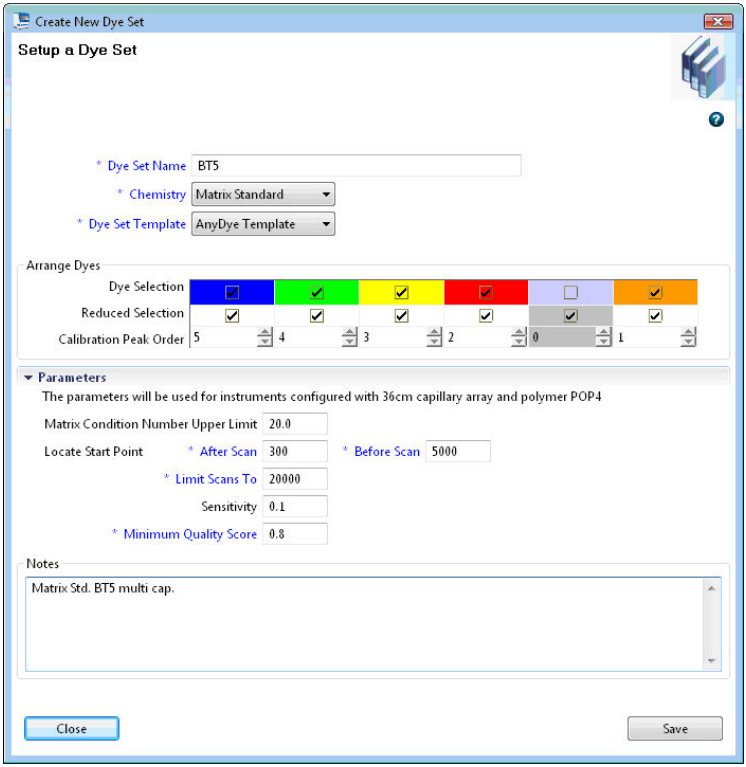


Fig. 6 Setup for dye set BT5

Performing a spectral calibration run

Once the multi-well plate containing the spectral calibration mixture is placed in the autosampler tray the spectral calibration process can be started.

1. To access the Spectral Calibration screen, select **Maintenance** on the Dashboard of the 3500 Series Data Collection software.
2. The number of wells in the spectral calibration plate and their location in the instrument must be specified.
3. Select **Matrix Standard** as a chemistry standard and **BT5** for dye set (defined before).
4. Enable **Allow Borrowing** (Optional).
5. Click **Start Run**.

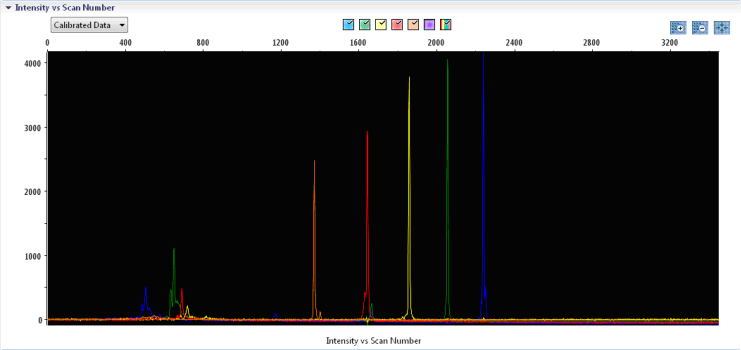


Fig. 7 Electropherogram of spectral calibration with matrix standard BT5 on an ABI 3500

Matrix check

- The quality value (**Q value**) of each capillary must be greater than 0.8 and the condition number range (**C value**) must be between 1 and 20
- Check the matrix samples for a flat baseline. As shown in the figure above, there should be five peaks with peak heights of about 1000-5000 RFU (Y-axis) in each matrix sample (optimal range: 2000-4000 RFU)
- A successful calibration will be displayed in green in **Overall** and for each capillary
- If all capillaries have passed the test, **Accept Results**
- If calibration failed, **Reject Results** and refer to the "spectral calibration troubleshooting" section in the Applied Biosystems 3500/3500xL Genetic Analyzer User Guide.

7.2 Sample preparation

Component	**Volume**
Hi-Di™ Formamide	12.0 µl
DNA Size Standard 550 (BTO)	0.5 µl
prepare 12 µl of the mix (formamide + DNA size standard) for all samples	
add 1 µl PCR product (dilute if necessary) or allelic ladder	

- Denaturation for 3 min at 95 °C
- Cool down to 4 °C and place samples on the autosampler tray

Since injections take place simultaneously on all capillaries, 8 or 24 samples must be pipetted on the plate of multi-capillary analyzers. If fewer samples are analysed empty positions need to be filled with 12 µl Hi-Di™ Formamide.

To ensure a reliable allelic assignment on multi-capillary analyzers, several ladders should be run.

Room temperature may influence the performance of PCR products on multi-capillary instruments, so that shoulder peaks or split peaks occur especially at low temperatures. Pay attention to keep ambient conditions as recommended by the instrument manufacturer. Optimal settings were reported >22 °C room temperature.

Signal intensities

Options to increase the signal intensity:

- Reduce the volume of the DNA Size Standard 550 (BTO) to peak heights of about 500 relative fluorescent units (RFU)
- Purify the PCR products before starting the analysis

7.3 Setting up a run

For the first run using the Mentype® **AMLplex**QS you will need to setup a number of protocols within the 3500 Series Data Collection Software.

Create Instrument protocol

- Go to **Library** and select **Analyze / Instrument protocol** and click **Create**
- Change the parameters according the table below

Instrument protocol for Mentype® AMLplexQS

Parameter	**Set up**
Application Type	HID / Microsatellite
Capillary Length	*Default*
Polymer	*Default*
Dye Set	BT5
Run Module	*Default*
Protocol Name	e.g. Mentype AMLplexQS
Oven Temperature [°C]	*Default*
Run Voltage [kV]	*Default*
Injection Voltage [kV]	**3.0**
Run Time [s]**	**1560**
PreRun Time [s]	*Default*
Injection Time [s]*	**10**
Data Delay Time [s]	*Default*
Advanced Options	*Default*

* Deviating from the standard settings, the injection time may range between 1 and 20 s depending on the type of sample. If reference samples with very high signal intensities are recorded, a shorter injection time may be selected in order to avoid pull-up peaks. For samples with low DNA content or critical patient samples an injection time of up to 20 s may be necessary.

** Depending on the analysis conditions, the run time for Mentype® **AMLplex**QS should be modified in order to analyse fragments with lengths of up to **550 bp**.

- Click on **Save** to confirm the settings

Create Size Standard

- Go to **Library** and select **Analyze / Size Standards** and click **Create**
- Change the parameters according the table below

Parameter	Set up
Size Standard	BTO_550
Dye Color	Orange

The DNA Size Standard 550 (BTO) should be used with the following lengths of fragments: **60**, **80**, **90**, **100**, **120**, **140**, **160**, **180**, **200**, **220**, **240**, **250**, **260**, **280**, **300**, **320**, **340**, **360**, **380**, **400**, **425**, **450**, **475**, **500**, **525**, and **550** bp.

- Click on **Save** to confirm the settings

Create QC (Size Calling) Protocol

- Go to **Library** and select **Analyze / QC (Size Calling)** and click **Create**
- Change the parameters according the table below

Parameter	Set up
Protocol Name	enter a name
Size Standard	BTO_550 (from above)
Sizecaller	Size Caller v.1.1.0

- Go to **Analysis Settings / Peak Amplitude Threshold** and **disable purple**. All other colors should be enabled
- Keep all other settings as Default
- Click on **Save** to confirm the settings

Create an Assay

- Go to **Library** and select **Manage / Assays** and click **Create**
- Change the parameters according the table below

Parameter	Set up
Assay Name	e.g. Mentype AMLplex^{QS}
Color	Default
Application Type	HID
Instrument Protocol	e.g. Mentype AMLplex^{QS}
QC (Size Calling) Protocol	e.g. BTO_550
Genemapper Protocol	could be defined

- Click on **Save** to confirm the settings

Starting the run

- Place the prepared multi-well plate on the autosampler tray
- In the Dashboard of the Data Collection software, click **Create New Plate**
- Go **to Define Plate Properties** and select **Plate Details**
- Change the parameters according the table below

Plate Details

Property	**Set up**
Name	e.g. Mentype AMLplex^{QS}
Number of Wells	96 or 384
Plate Type*	HID
Capillary Length	**36cm**
Polymer	**POP4**

- Click **Assign Plate Contents** to confirm the settings
- Define well position of each sample or ladder for data collection and processing by entering sample names
- Assign an **Assay** (required), a file name conventions, and a result group to all named wells in the plate
- Click **Link the plate for Run** and enter Run Name
- Click **Start Run**

8. Analysis

For general instructions on automatic sample analysis, please refer to the *GeneScan* or *GeneMapper® ID/ID-X Software User's Manual*.

Note: Within the Mentype® **AMLplex^{QS}** the red panel should be faded out.

Finding the exact lengths of the amplified products depends on the device type, the conditions of electrophoresis, as well as the DNA size standard used. Therefore, determining the size should be based on evenly distributed references. The DNA Size Standard 550 (BTO) shall thus be used with the following lengths of fragments: **60**, **80**, **90**, **100**, **120**, **140**, **160**, **180**, **200**, **220**, **240**, **250**, **260**, **280**, **300**, **320**, **340**, **360**, **380**, **400**, **425**, **450**, **475**, **500**, **525**, and **550 bp**.

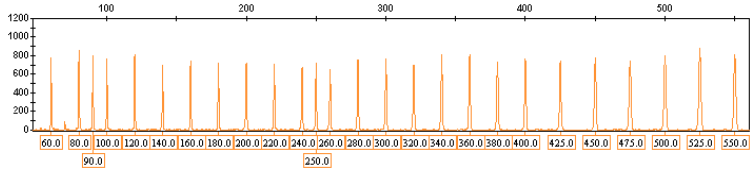


Fig. 8 Electropherogram of the DNA Size Standard 550 (BTO), fragments with lengths in bp.

Note: The provided template file for the DNA size standard SST-BTO_60-550bp can be applied for the evaluation and analysis of the Mentype® **AMLplex^{QS}** using the GeneMapper® ID/ID-X Software.

8.1 Biotype® template files

Allocation of fusion gene transcripts and variants should be carried out with suitable analysis software, e.g. GeneMapper® ID/ID-X software in combination with the Mentype® **AMLplex**^{QS} template files from Biotype. The Biotype® template files with the respective manual are available on our homepage (www.biotype.de) for download or as CD-ROM on request.

Recommended Biotype® templates for GeneMapper® ID/ID-X Software are:

Panels	AMLplex_Panels_v1	or higher version
BinSets	AMLplex_Bins_v1	or higher version
Size Standard	SST-BTO_60-550bp	
Analysis Method	AMLplex_HID_310_200rfu	recommended
	AMLplex_HID_3130_200rfu	recommended
Plot Settings	PlotsBT5_4dyes	
Table Settings	Table for 10 Alleles	
	Table für 22 Alleles	

Panels and BinSets have to be used at any time, whereas other template files are optional.

Important Note: Import and allele calling with provided template files is only guarantied using GeneMapper® ID/ID-X software. If GeneMapper® software is applied you may experience import problems using some template files. You may have to adjust Panels and Bins with one ore more runs of the allelic ladder on your specific instrument setup. Contact us for support (support@biotype,de).

General procedure for the analysis

1. Check the DNA size standard
2. Check the allelic ladder
3. Check the positive control
4. Check the negative control
5. Analyse and interpret the sample data

8.2 Controls

The Mentype® **AMLplex**QS PCR Amplification Kit includes a cDNA Control that represents the following aberrations:

Table 3. Allocation with the Mentype® **AMLplex**QS

cDNA from cell culture*	Aberration
KASUMI-1 (Asou et al. 1991)	AML1-ETO

*Cell culture for preparation of cDNA was purchased from DSMZ - Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany.
Use of provided cDNA is restricted to Mentype® **AMLplex**QS only.

8.3 Fragment lengths and aberration variants

Table 4 shows fragment lengths of the individual variants that refer to the DNA Size Standard 550 (BTO). All analyses have been performed on an ABI PRISM® 3130 Genetic Analyzer with POP-4® polymer. Different analysis instruments, DNA size standards or polymers may result in different fragment lengths. Due to instrument specific differences individual fine tuning of actually measured fragment sizes (home-based apparatus) is recommended.
In addition, a visual alignment with the allelic ladder is also recommended.

Scaling

Horizontal: 55-550 bp
Vertical: Depending on signal intensity

Figure 9

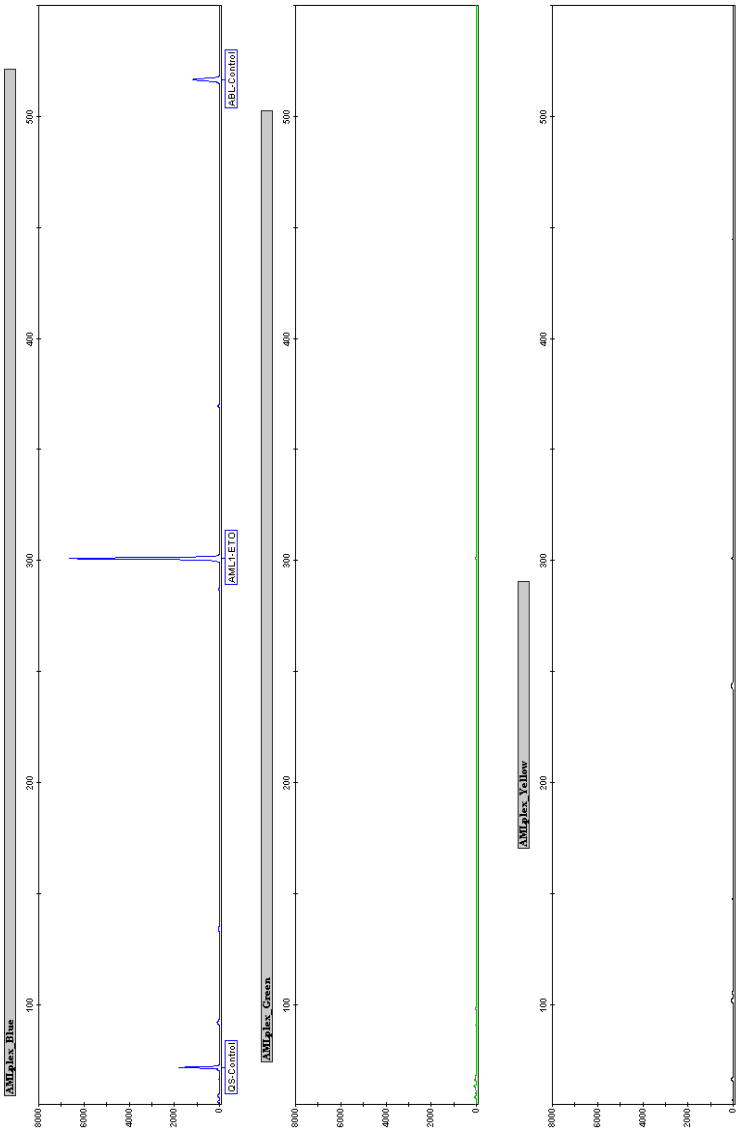


Fig. 9 Electropherogram of the Mentype® **AMLplex**^{QS} using 250 ng cDNA Control KASUMI-1. Analysis was performed using the ABI PRISM® 3130 Genetic Analyzer and the DNA Size Standard 550 (BTO). Assignment was done with GeneMapper® ID Software and the Mentype® **AMLplex**^{QS} template file.

Figure 10

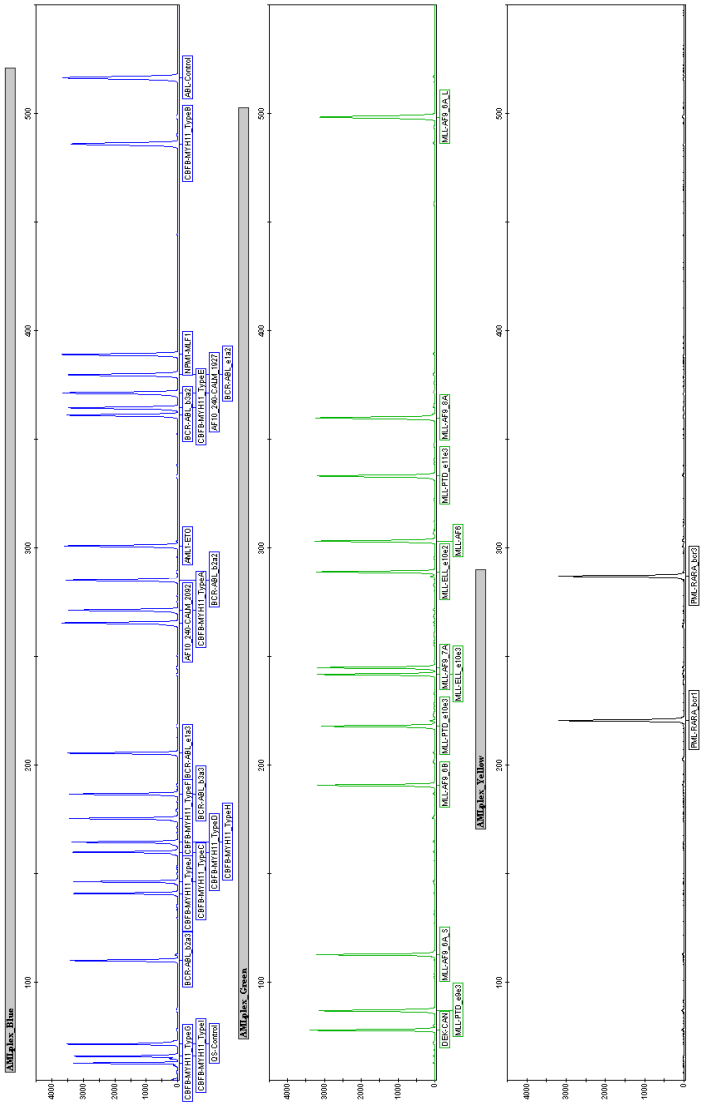


Fig. 10 Electropherogram of the Mentype® **AMLplex**^{QS} allelic ladder. Analysis was performed using the ABI PRISM® 3130 Genetic Analyzer and the DNA Size Standard 550 (BTO). Assignment was done with GeneMapper® ID Software and the Mentype® **AMLplex**^{QS} template file.

Table 4. Fragment lengths of the Mentype® **AMLplex**QS allelic ladder measured using the ABI PRISM® 3130 Genetic Analyzer with POP-4® polymer.

Panel/Variants	Size [bp]*	Others	Panel/Variants	Size [bp]*	Others
AMLplex Blue			**AMLplex Green**		
CBFB-MYH11_TypeG	63		DEK-CAN	78	
CBFB-MYH11_TypeI	66		MLL-PTD_e9e3	87	
QS-Control	72		MLL-AF9_6A_S‡	113	
BCR-ABL_b2a3	110		MLL-AF9_6B	191	
CBFB-MYH11_TypeJ	140		MLL-PTD_e10e3	218	
CBFB-MYH11_TypeC	146		MLL-ELL_e10e3	242	
CBFB-MYH11_TypeD	160		MLL-AF9_7A	245	
CBFB_MYH11_TypeH	165		MLL-ELL_e10e2	289	
CBFB_MYH11_TypeF	175		MLL-AF6	303	
BCR-ABL_b3a3	186		MLL-PTD_e11e3	333	
BCR-ABL_e1a3	206		MLL-AF9_8A	360	
AF10_240-CALM_2092	265		MLL-AF9_6A_L‡	498	
CBFB-MYH11_TypeA	271				
BCR-ABL_b2a2	285		**AMLplex Yellow**		
AML1-ETO	301		PML-RARA-bcr1	220	
BCR-ABL_b3a2	361		PML-RARA_bcr3	288	
CBFB-MYH11_TypeE	365				*PML-RARA_bcr2***
AF10_240-CALM_1987	371				
BCR-ABL_e1a2	380				
NPM1-MLF1	389				
CBFB-MYH11_TypeB	486				
ABL-Control	518				

* rounded to integer

** Although this variant is detectable with Mentype® **AMLplex**QS primers, the varying length of the amplicon (apprx. 173 bp) prevents automated allocation.

‡ Two amplicons for variant MLL-AF9_6A

9. Interpretation of results

As mentioned above, post PCR analysis and automatic allele assignment with suitable analysis software ensure a precise and reliable discrimination of fusion gene transcripts and variants.

Detection limit

Applying plasmids, experimental data showed that 1000 copies resulted in peaks-heights > 200 RFU.
Please note that Mentype® **AMLplex**^{QS} was designed, validated and certified as a screening tool for subtype classification of AML. This application is not suited to quantify copy numbers or monitor Minimal Residual Disease (MRD).

Pull-up peaks

Pull-up peaks may occur if peak heights are outside the linear detection range of the instrument, or if an incorrect matrix was applied. They appear at positions of specific peaks in other color channels, typically with lower signal intensities.

Template-independent addition of nucleotides

Because of its terminal transferase activity, the Multi Taq DNA Polymerase tends to add an adenosine radical at the 3'-end of the amplified DNA fragments. The artefact peak is one base shorter than expected (-1 bp peaks). All Biotype® primers are designed to minimise these artefacts. Artefact formation is further reduced by the final extension step of the PCR protocol at 68°C for 10 minutes. Peak height of the artefact correlates with the amount of cDNA. Laboratories should define their individual limits for analysis of the peaks.

Artefacts

Room temperature may influence the performance of PCR products on multi-capillary instruments, shoulder peaks or split peaks occur. Furthermore, automated assignment could be influenced in some cases. If these effects occur we recommend injecting the sample again at higher room temperature and maybe using more than one allelic ladder sample per run.

Influence of polymer types

The Mentype® **AMLplex**^{QS} kit was validated and certified for the analysis on POP-4® polymer. The use of other polymers (e.g. POP-7™ or POP-6™) might influence the run behaviour of specific PCR products. Furthermore background noise might increase through different behaviour of free fluorescent dyes.

10. References

Asou H, Tashiro S, Hamamoto K, Otsuji A, Kita K, Kamada N (1991) Establishment of a human acute myeloid leukemia cell line (Kasumi-1) with 8;21 chromosome translocation. *Blood 77(9): 2031-2036*.

Beillard E, Pallisgaard N, van der Velden VHJ, Bi W, Dee R, van der Schoot E, Delabesse E, Macintyre E, Gottardi E, Saglio G, Watzinger F, Lion T, van Dongen JJM, Hokland P, Gabert J (2003) Evaluation of candidate control genes for diagnosis and residual disease detection in leukemic patients using ‚real-time' quantitative reverse-transcriptase polymerase chain reaction (RQ-PCR)- a Europe against cancer program. *Leukemia 17:2474-2486.*

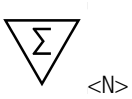
Van Dongen JJM, Macintyre EA, Gabert JA, Delabesse E, Rossi V, Saglio G, Gottardi E, Rambaldi A, DOtti G, Griesinger F, Parreira A, Gameiro P, Gonzalez Diaz M, Malec M, Langerak AW, San Miguel JF, Biondi A (1999) Standardized RT-PCR analysis of fusion gene transcripts from chromosome aberrations in acute leukemia for detection of minimal residual disease - Report of the BIOMED-1 Concerted Action: Investigation of minimal residual disease in acute leukemia. *Leukemia 13:1901-1928.*

11. Explanation of Symbols

Manufacturer

Date of manufacture

Batch code



Contains sufficient reagents for <N> tests

Consult instructions (handbook) for use

Use by

Temperature limitations

Catalogue number

In-Vitro-Diagnostics

Notes

Notes

Notes